FIG. 1

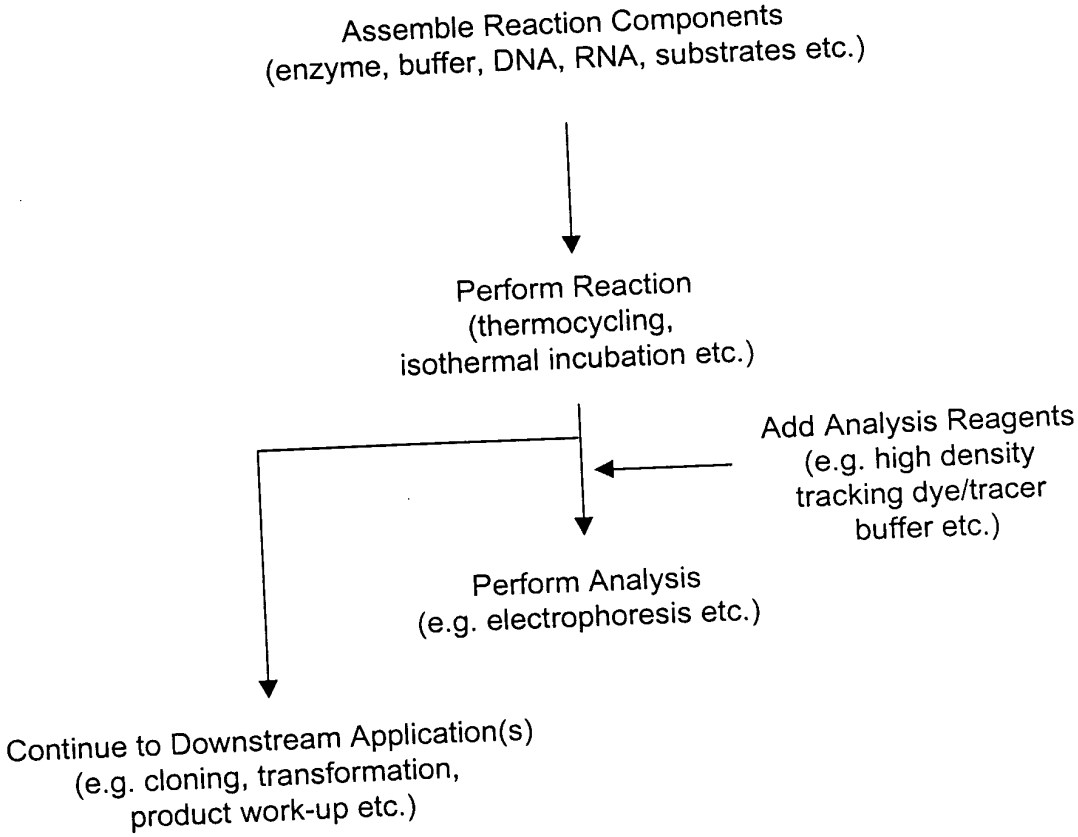


FIG. 2

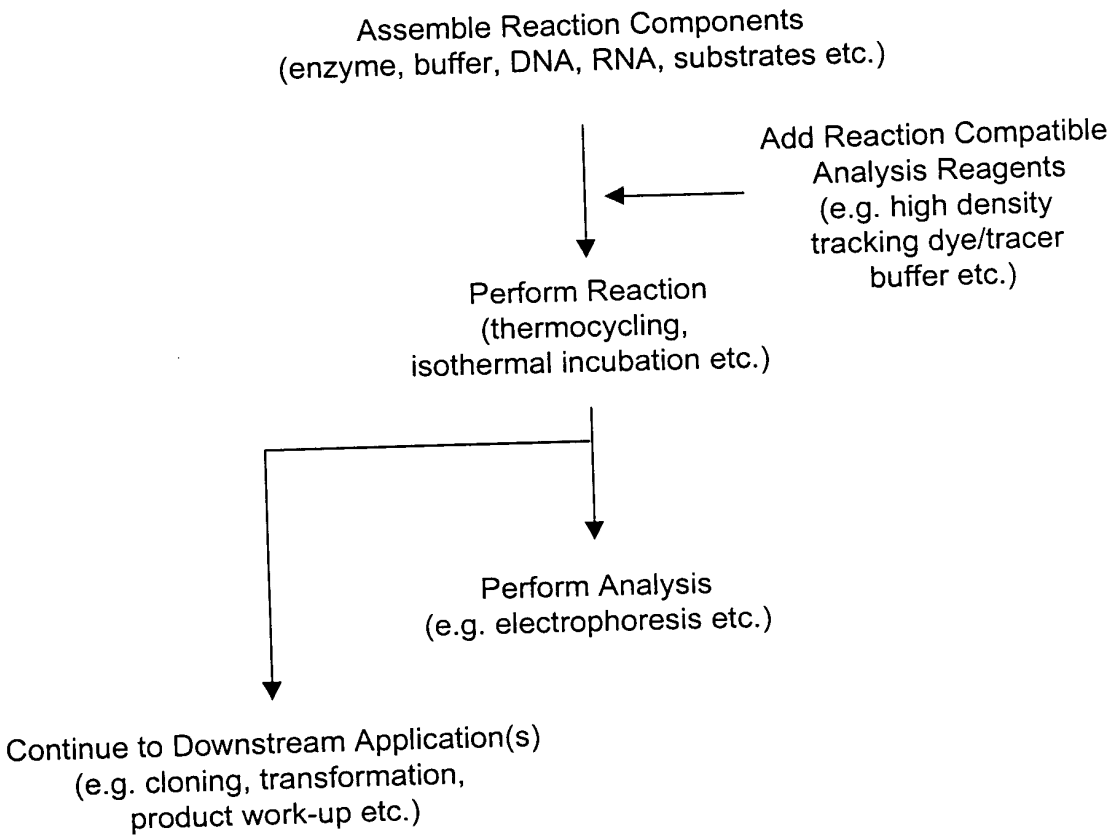


FIG. 3

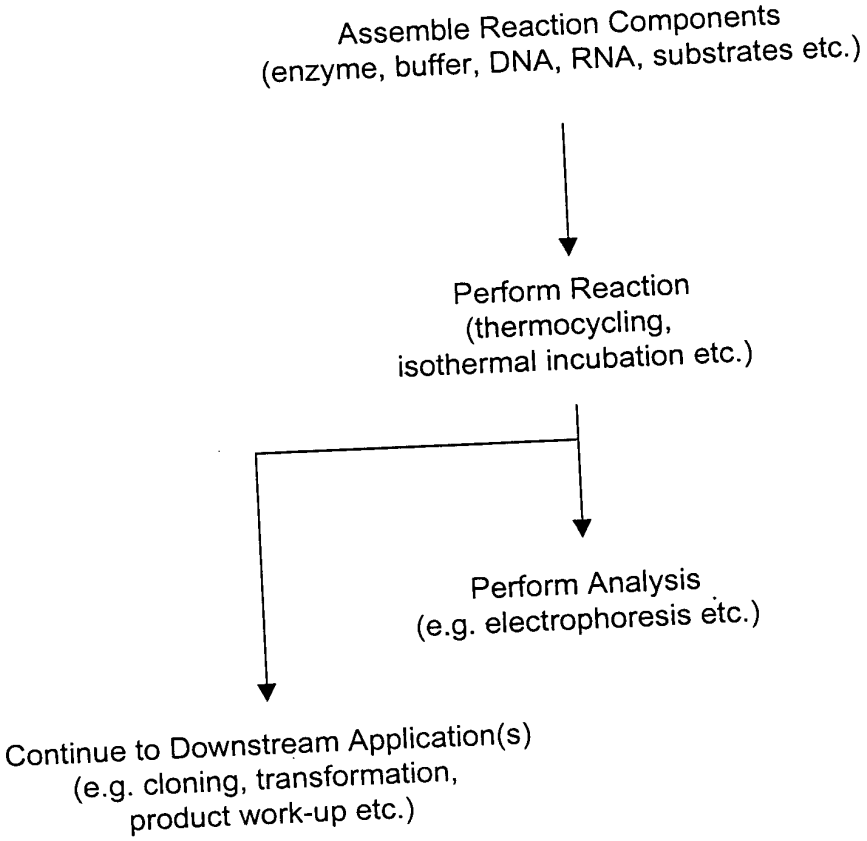


FIG. 4

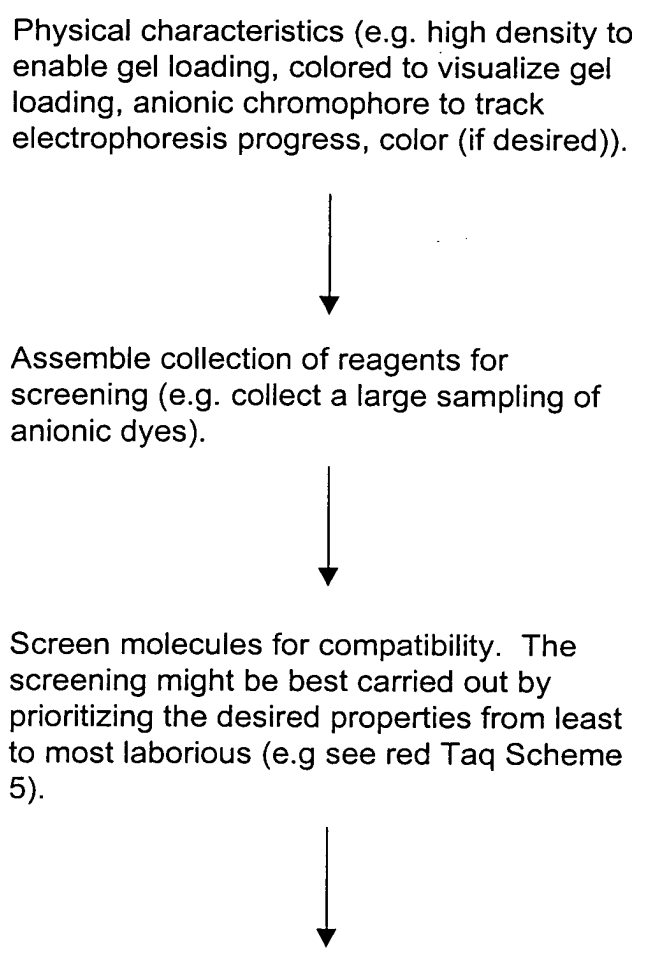


FIG. 5

Physical characteristics.
High density to enable gel loading- formulate enzyme dilute enough so that enough glycerol will be contained in a 2.5 unit per 50 microliter reaction.
Colored to visualize gel loading and act as a tracking dye- red anionic water and ethanol soluble dyes were sought.

↓

Assemble collection of reagents for screening - 40 anionic "red" (lambda max =450-550) dyes were selected as candidates.

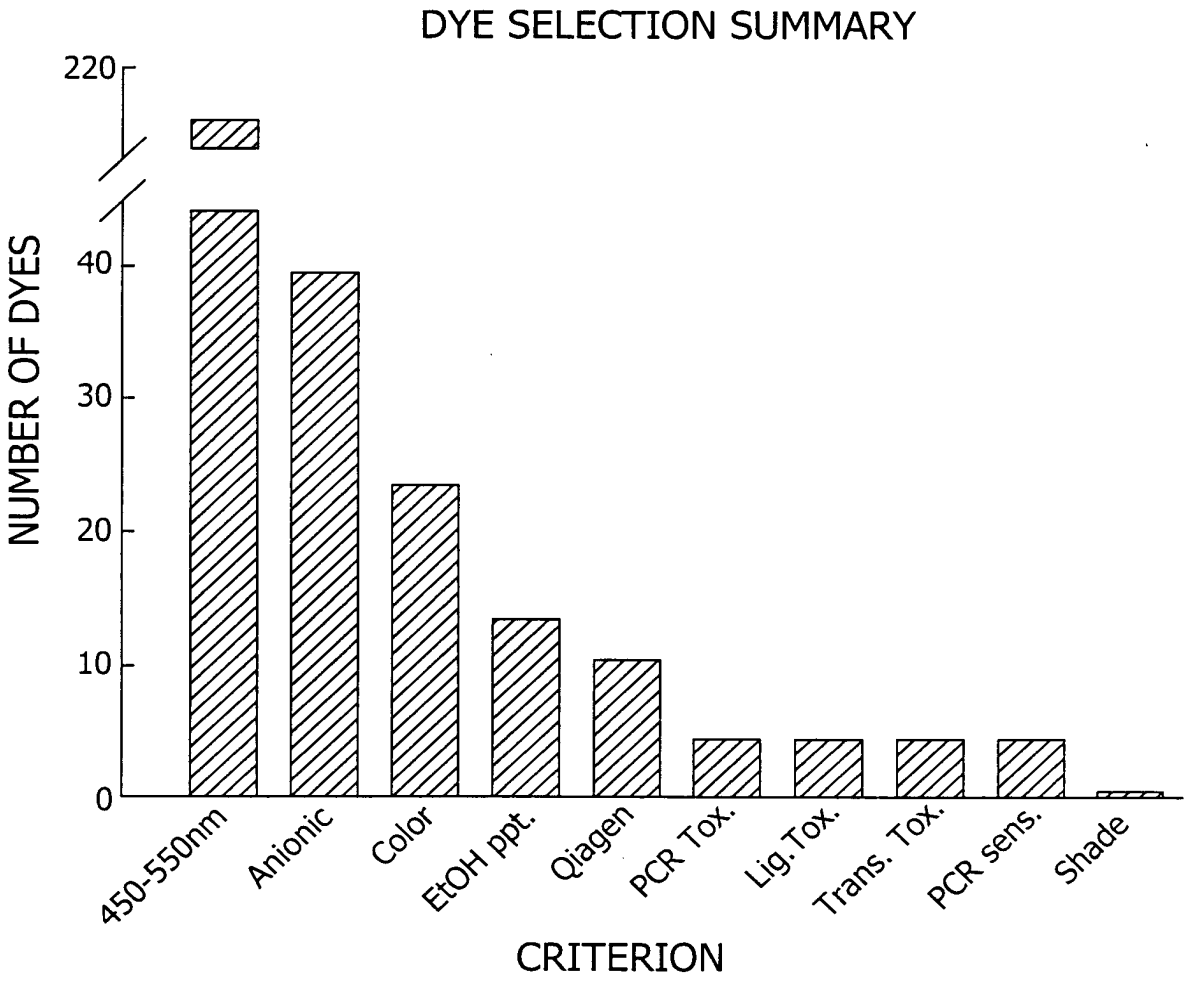
↓

Screen molecules for compatibility-summarized in Figure 1 . The dyes were scruinized in the order:
1. Color (too yellow or purple thrown out)
2. Ethanol precipitation (colored DNA pellets thrown out)
3. Chaotropic salt/silica DNA purification (colored product thrown out)
4. PCR toxicity (Figure 2, low or no yield thrown out).
5. Ligase toxicity (Figure 3, low or no yield thrown out).
6. Transformation toxicity (Figure 4, low or no yield thrown out).
7. Remaining dyes more or less equivalent, submit to marketing for color selection.

↓

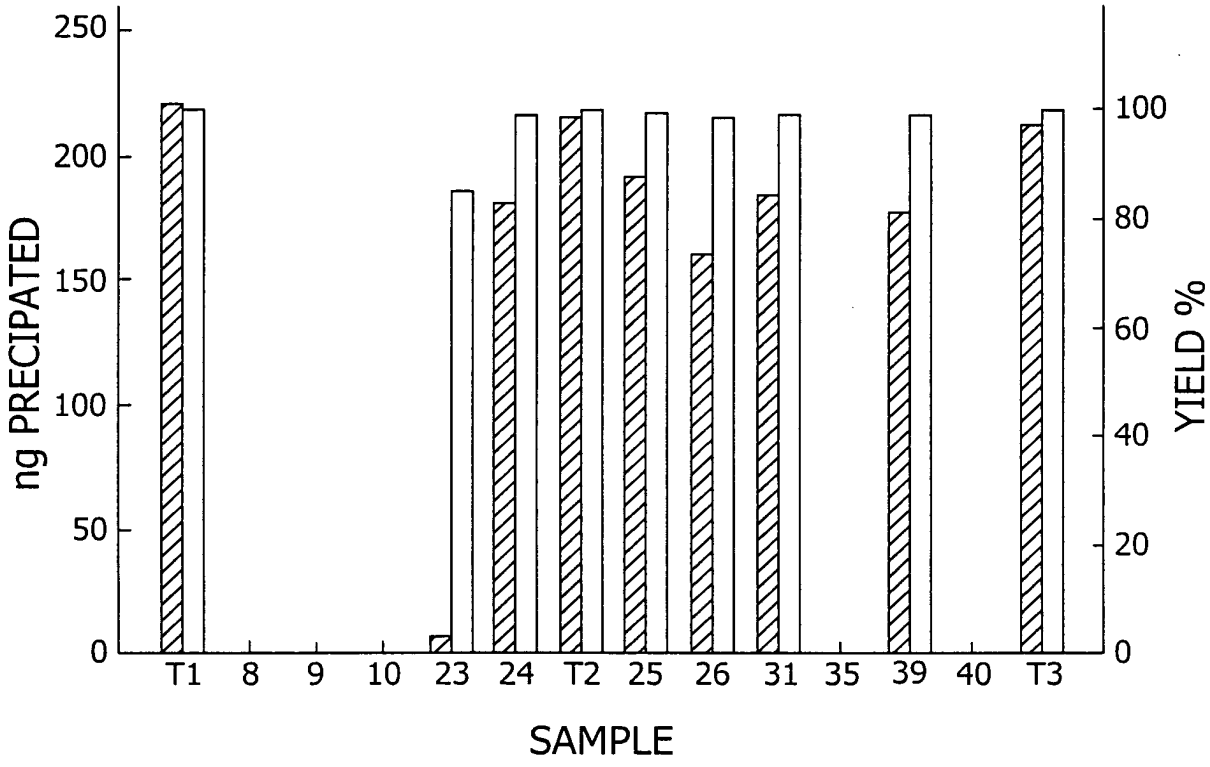
Formulate reagent- 80% acid red 1, 20% acid violet 5 (100%= absorbance of acid red 1 at lambda max + absorbance of acid violet 5 at lambda max.) to absorbance total =300 in Taq DNA polymerase at 1 unit per microliter in Taq storage buffer (20 mM Tris- HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Igepal CA- 630, 50% glycerol)
Characterize reaction products-product yields low relative to absence of dye reactions (Figure 5). Dye purity (Figure 6) and counter ion identity (Figure 7) investigated for PCR toxicity/compatibility. Purified Mg acid red 1 and Mg Acid violet 5 found to be satisfactory.
Optimize other reaction components- Mg dyes contribute approximately 0.4 mM "free" Mg to PCR (Figure 8), 10X PCR buffer adjusted from 15 to 11 mM to accommodate Mg dye contribution. **Final characterization-** quality: gel (Figure 9), quantity (Figure 10). Limitations- will test with a panel of restriction enzymes, does not impact fluorescent sequencing.

FIG. 6



430-570 nm-visible absorption max.
Anionic-anionic dyes
Color-not too yellow/orange or purple
EtOH ppt. did not co-precipitate with DNA
Chaotropic salt/silica purifacation (Qiagen PCR columns)-isolated DNA was colorless
PCR Tox.-little impact upon ^{32}P PCR product yield
Lig. Tox.-little to no effect upon ligation of lambda PstI fragments
Trans. Tox.-no effect upon ligation/transformation of EcoRI-pUC19
PCR Sens.-amplification similar to no dye as a function of template concentration.
shade-marketing

FIG. 7



T1, T2 and T3= Taq controls (no dye) as in block and precipitation, numerals are dye number.
per cycle yield calculated assuming $y_{20} = y_1^{20}$ where y is the 20 cycle yield (measured)and y_1 is the per cycle yield.

Dye	y(20%)	y1(%)
23	9.04	85.3
24	84.1	99.1
25	88.9	99.4
26	74.6	98.5
31	85.6	99.3
39	82.4	99.0

FIG. 8

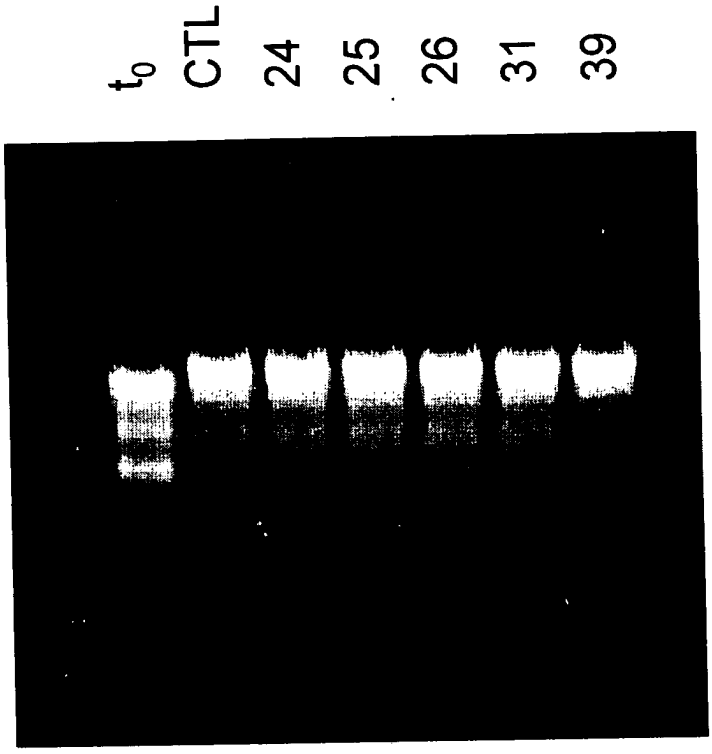


FIG. 9

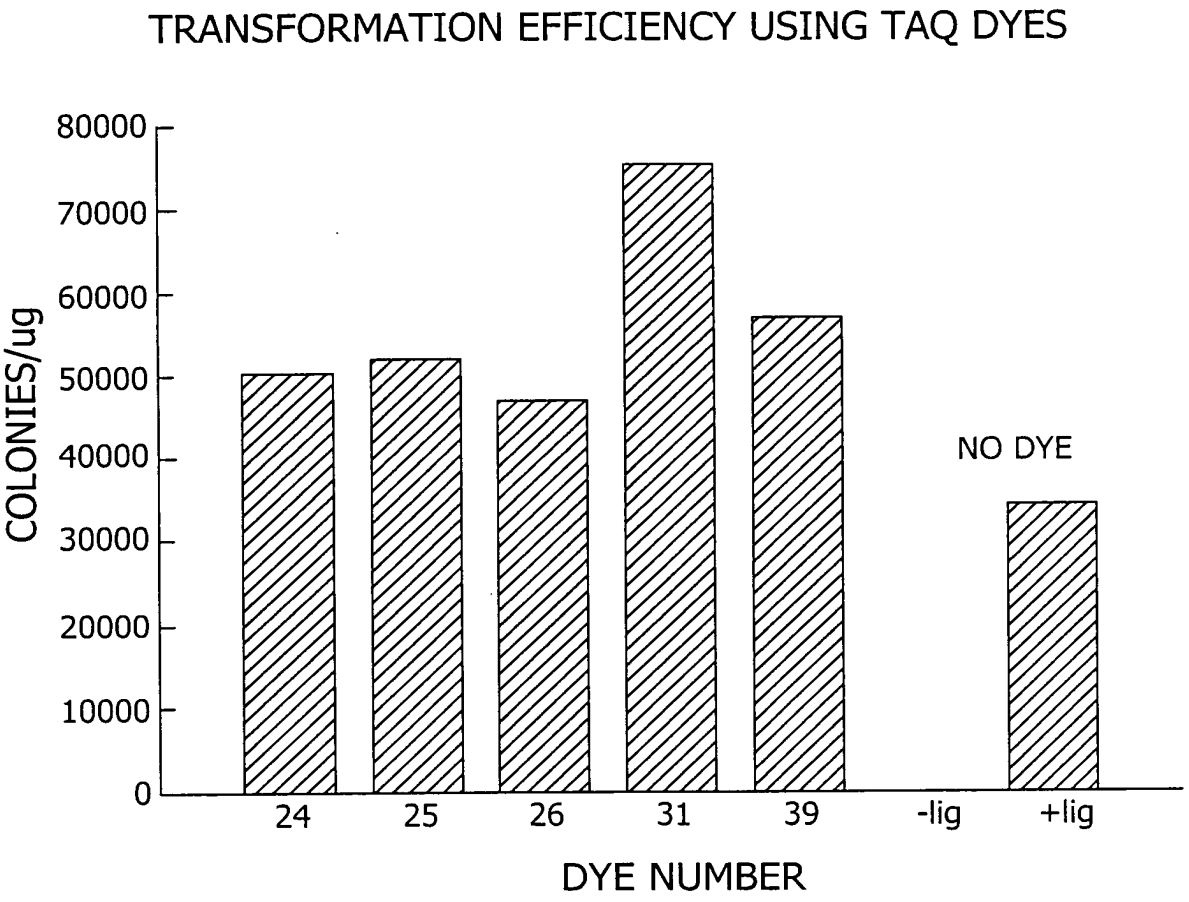


FIG. 10

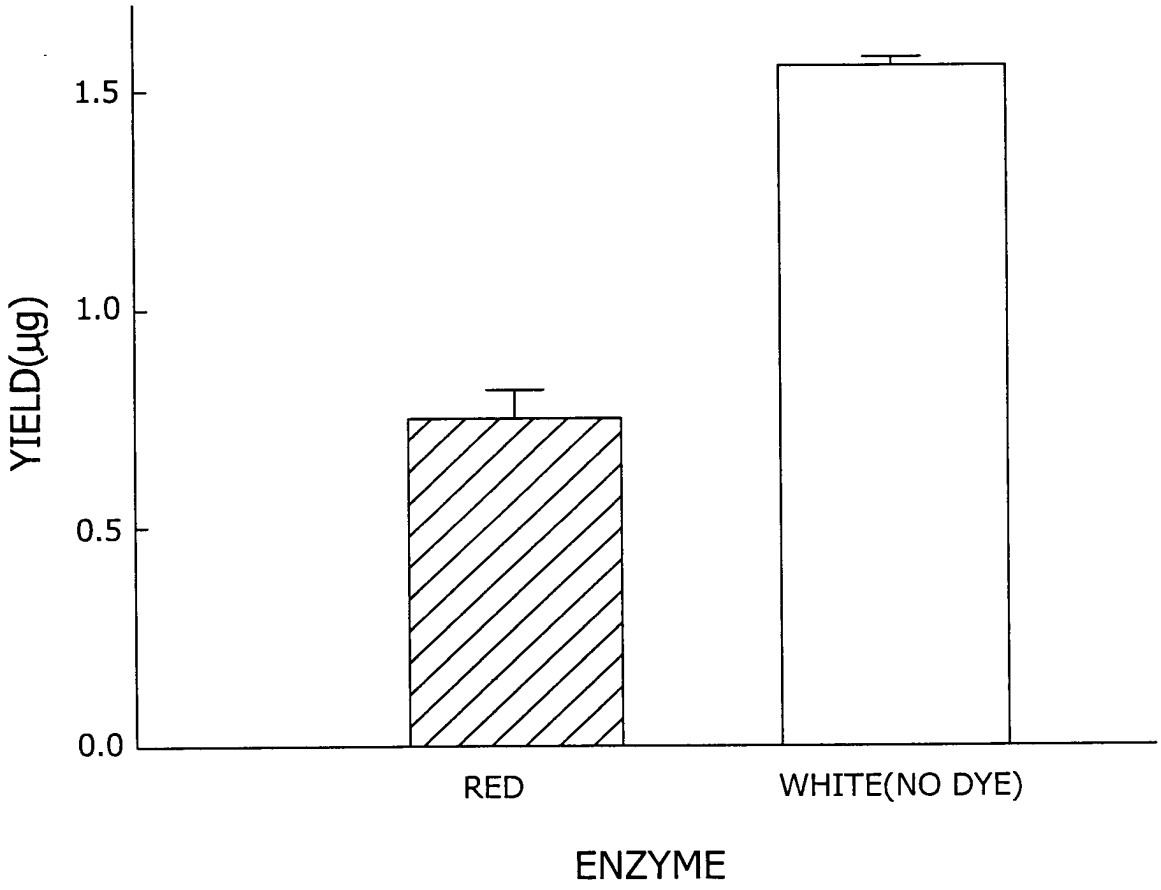


FIG. 11

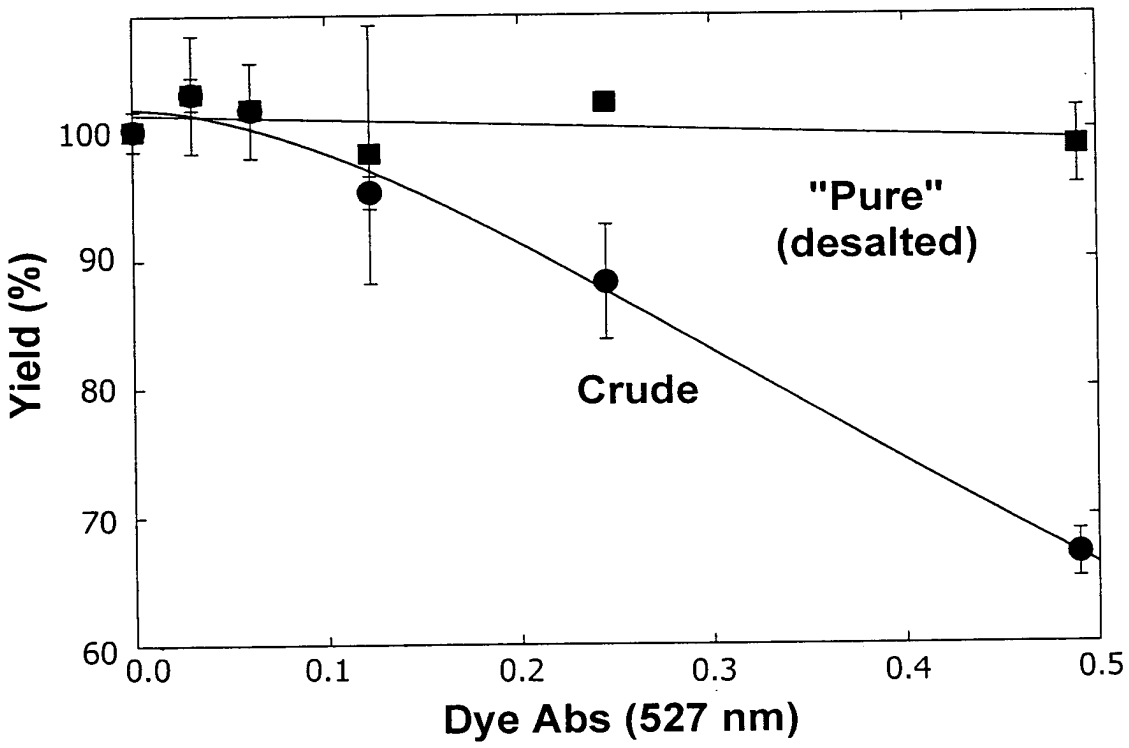


FIG. 12A

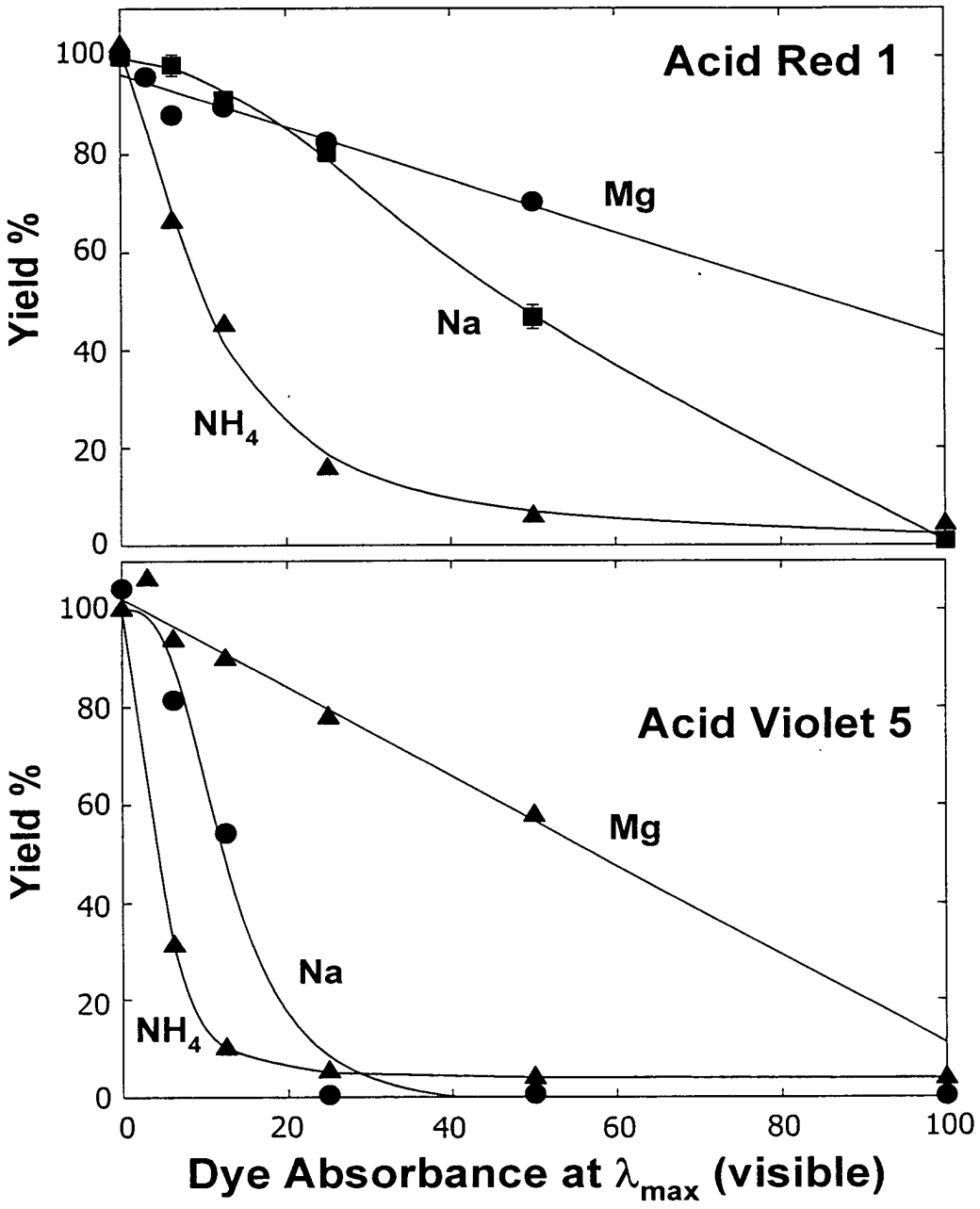


FIG. 12B

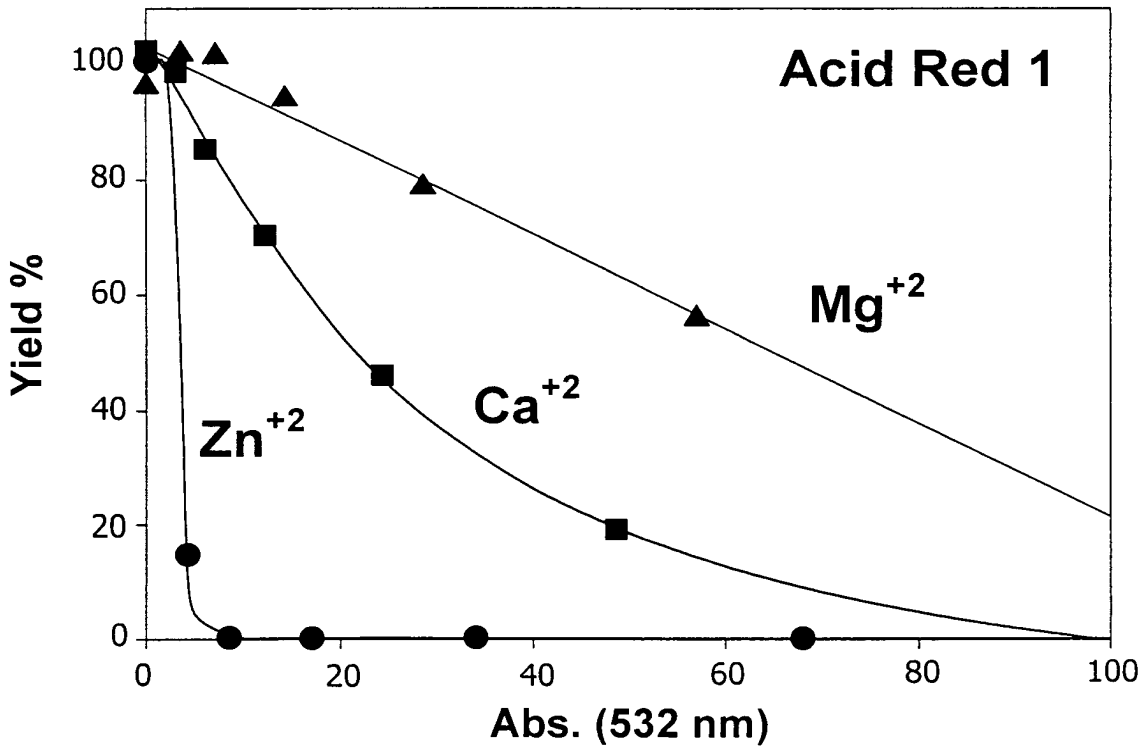


FIG. 13

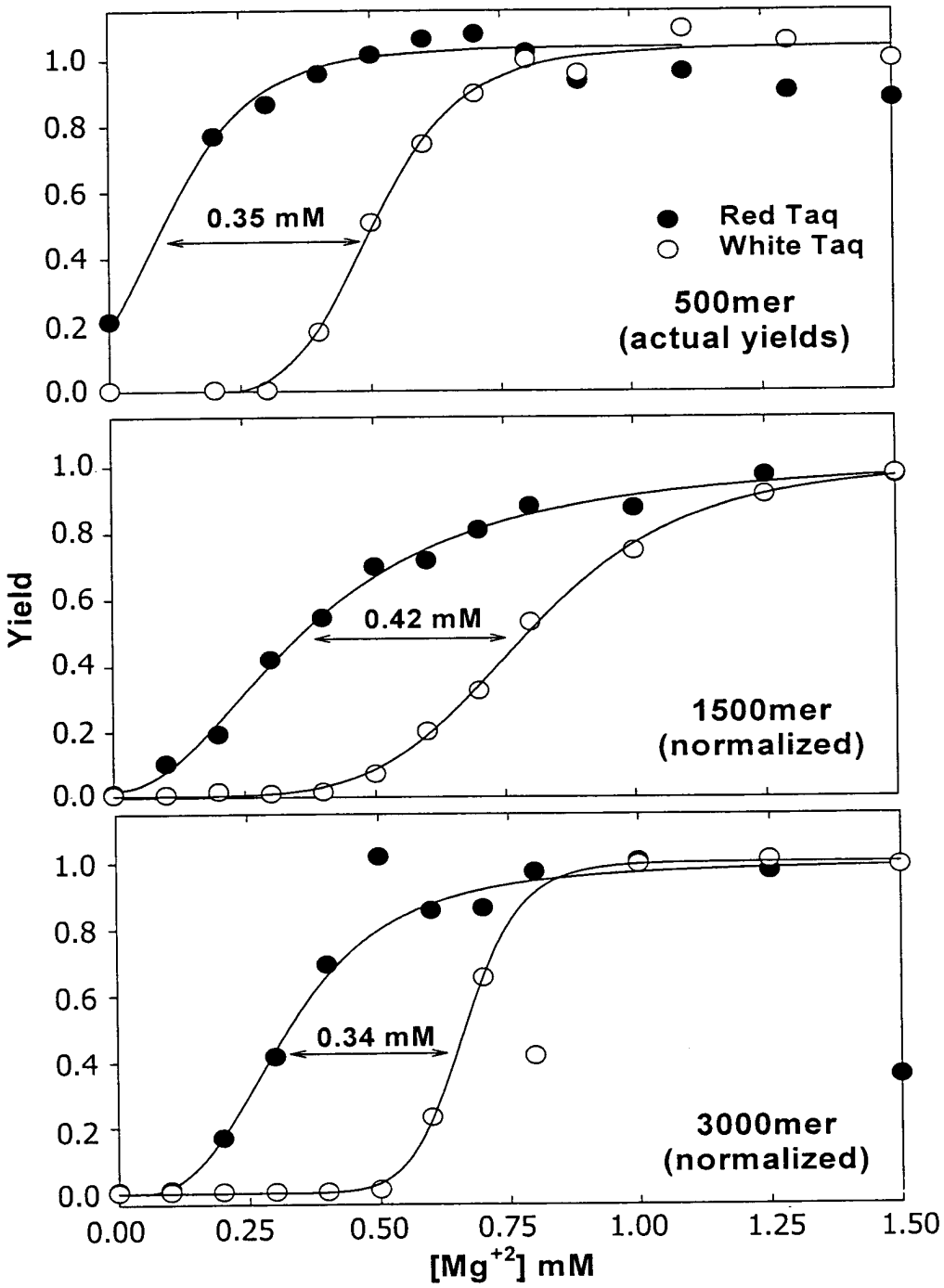


FIG. 14

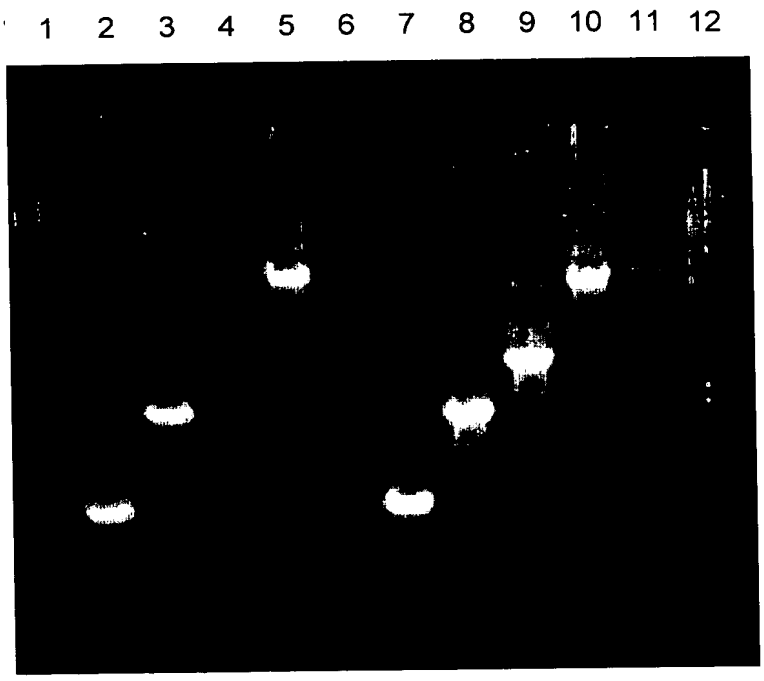


FIG. 15

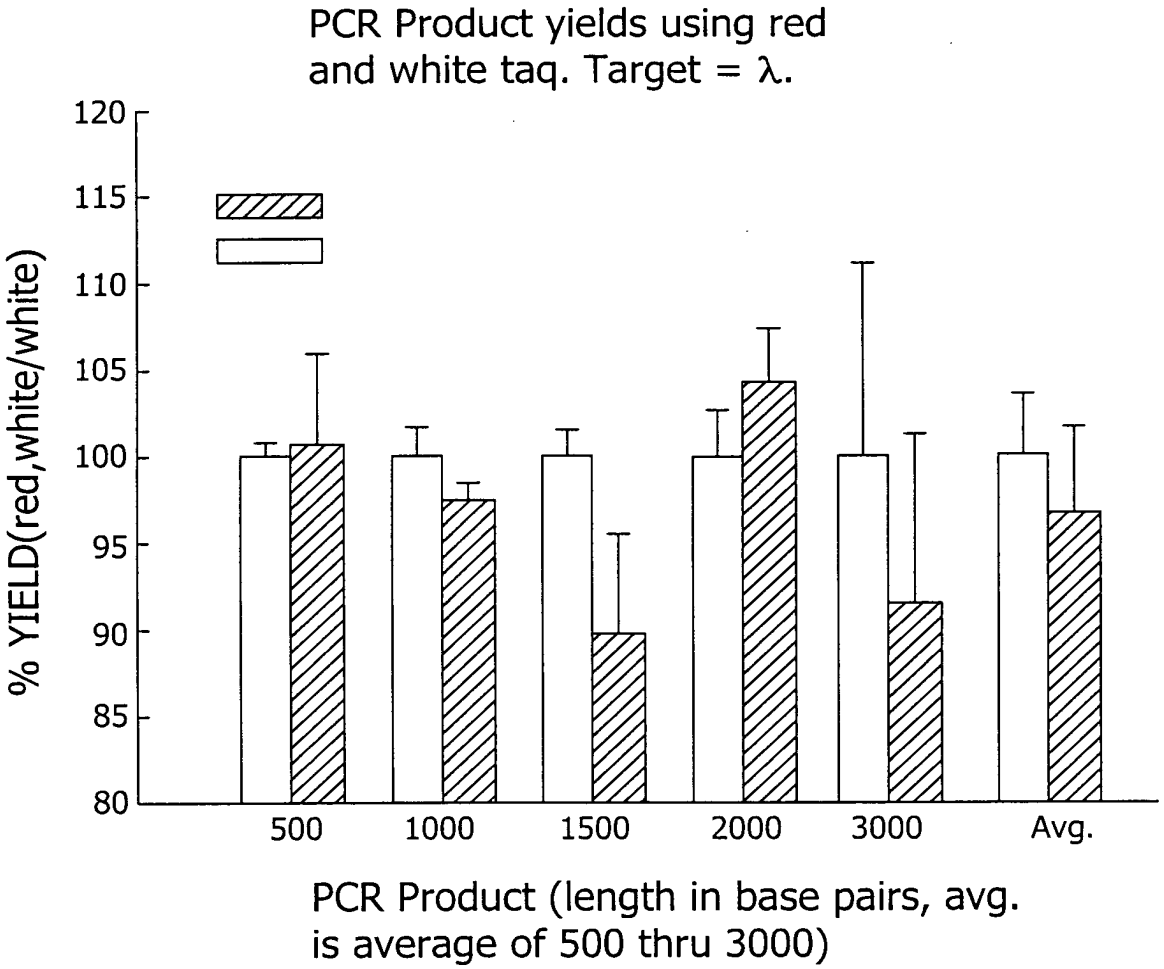


FIG. 16

FIG. 17